

1 **Photodynamic therapy and diagnosis: Principles and comparative aspects**

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19 **Abstract**

20 Photodynamic therapy (PDT) is an evolving method of treating superficial tumours that is
21 non-invasive and carries minimal risk of toxicity. PDT combines tumour-selective photosensitiser
22 dyes, tissue oxygen and targeted illumination to generate cytotoxic reactive oxygen species (ROS)
23 within the tumour. In addition to directly acting on tumour cells, PDT damages and restricts tumour
24 microvasculature, and causes a local inflammatory response that stimulates an immune response
25 against the tumour. Unlike surgery or radiotherapy the surrounding extracellular matrix is
26 unaffected by PDT, thus tissue healing is excellent and PDT seldom scars. This, combined with the
27 ease of light application, has made PDT a popular treatment for cancers and pre-cancers in humans.
28 Moreover, because photosensitiser dyes are fluorescent and selectively accumulate in tumour
29 tissues, they can additionally be used to visualise and discriminate tumour from normal tissues,
30 thereby improving the accuracy of tumour surgery.

31

32 In veterinary practice, PDT has been used successfully for treatment of superficial squamous
33 cell carcinoma of the feline nasal planum; urinary tract, bladder and prostate neoplasia in dogs; and
34 for equine sarcoids. The purpose of this article is to make a comparative review of the current
35 literature on PDT in human and veterinary medicine, to provide a basis for future development of
36 PDT in veterinary medicine.

37

38 **Key words**

39 Photodynamic therapy; photodiagnosis, cancer, comparative and veterinary.
40
41

42 **Introduction**

43 Photodynamic therapy (PDT) involves administration of a photosensitiser drug, or a pro-
44 drug, which selectively accumulates in target cells, followed by local illumination of the lesion with
45 visible light (Luksiene, 2003; Wachowska et al., 2011). It is a minimally invasive therapeutic
46 technique used in the management of various cancerous and pre-malignant diseases. The
47 photosensitiser can also be visualised in tumour cells using an appropriate set of imaging filters to
48 provide a means of tumour detection (Hefti et al., 2010, Mowatt et al., 2011, , Nguyen and Tsien
49 2013, Allison 2016).

50

51 In addition to cancer treatment, PDT has been used for the treatment of microbial infections
52 (Kharkwal et al., 2011, Sharma et al., 2012, Wardlaw et al., 2012), including veterinary applications
53 in dogs (Fabris et al., 2014) and sheep (Sellera et al., 2016). PDT has also been used for light-
54 triggered uptake of pharmaceuticals that would otherwise become entrapped and destroyed within
55 cellular endosomes (photochemical internalisation, PCI; reviewed by Selbo et al., 2015 and Madsen
56 2016). However, the focus of this review will be on the uses of PDT in cancer treatment and
57 diagnosis.

58

59 The origins of PDT can be traced back to ancient Egypt, where photosensitizing plant
60 pigment extracts were applied to the skin and exposed to sunlight, as a treatment for psoriasis
61 (Daniell and Hill 1991). The use of PDT for treatment of various human skin cancers was first
62 investigated in the 1970's by Dougherty et al (1978). Dougherty's use of a haematoporphyrin
63 derivative was based on pioneering work of Policard et al., (1924) who demonstrated that
64 porphyrins were preferentially distributed into malignant rather than normal tissues. The technique
65 was slow to gain acceptance because the 'first generation' photodynamic agents were slow to clear
66 from normal cells with the result that treated human patients had to remain out of bright light

67 (e.g.sunlight) for several weeks to avoid severe skin reactions. However, the potential for the
68 technique in treating locally advanced carcinomas of the head and neck (Wile et al., 1984), bladder
69 (Misaki et al., 1983), oesophagus and bronchus (Cortese and Kinsey 1984) outweighed this caveat
70 and stimulated further research.

71

72 The availability of haematoporphyrin derivatives with faster tissue clearance times
73 stimulated more interest in PDT and numerous human clinical trials have now been published
74 showing encouraging results with photosensitizing dyes administered topically or systemically
75 (orally or intravenously) or instilled into hollow organs (e.g. bladder). A limited number of
76 veterinary studies have been published, also showing promise. A previous review of PDT in
77 veterinary medicine was published in 2013 (Buchholz and Walt, 2013), since then further advances
78 have been made. The purpose of this review is to describe the basic principles of PDT and discuss
79 the clinical application of PDT in humans and animals.

80

81 **Fundamentals and mechanisms**

82 There are three basic requirements for PDT; (1) a compound with photosensitising
83 properties (photosensitizer, PS), (2) a source of visible light and (3) oxygen. The photosensitizer is a
84 chemical / dye that selectively accumulates in malignant tissues and can be activated by visible
85 light. Energy from the light-excited PS is transferred to oxygen molecules (O_2) to give reactive
86 oxygen species (ROS), notably singlet oxygen (1O_2) and superoxides, that damage biological
87 molecules, initiating a cascade of biochemical events culminating in damage and death of neoplastic
88 cells (Fig. 1) (Dougherty et al., 1998, Juzeniene et al., 2007). Increasing tissue oxygenation can lead
89 to increased ROS formation during PDT and improved outcomes (Maier et al., 2000).

90

The mechanisms by which different photosensitisers localise selectively in malignant tissues are complex and not fully understood. Physical factors, such as increased vascular permeability and poor lymphatic drainage in tumours, coupled with an affinity for proliferating endothelium likely contribute to their accumulation in tumours (Dougherty et al., 1998).

Three main processes by which ROS contribute to the destruction of tumours by PDT are direct cellular damage, indirect vascular shutdown and activation of immune response against tumour cells (Dougherty et al., 1998, Dolmans et al., 2003, Solban et al., 2006). Direct damage to tumour cells can result in cell death by both programmed (apoptotic) pathways and non-programmed (necrotic) pathways (Oleinick et al., 2002; Igney and Krammer 2002, Allison and Moghissi 2013a). Generally, when the light intensity is low, apoptotic death may be initiated (Agarwal et al., 1991, Allison and Moghissi 2013b). At higher light intensities, tumour cells are rapidly ablated by necrosis due to destruction of cellular and subcellular membranes. This also leads to release of cytokines and lysosomal enzymes (Henderson and Fingar 1987) causing damage to cells nearby, the bystander effect (Dahle et al., 1997, Allison and Moghissi, 2013a). Release of inflammatory mediators from the treated region stimulates activation of leucocytes including neutrophils and macrophages and significant tumour cell death occurs through these activated immune cells (Coutier et al., 1999; Gollnick et al., 2003, Castano et al., 2006). This observation has led to the development of combination therapies of PDT with immunotherapy, by including immunoadjuvants against tumour-specific epitopes (Qiang et al., 2008, Kleinovink et al., 2015).

PDT also mediates a vascular effect within tumours (McMahon et al., 1994, Abels, 2004). Neovascular tumour endothelial cells may accumulate higher levels of PS than normal endothelium (Debefve et al., 2011) and following PDT, microvascular collapse can be observed and can lead to severe and persistent post-PDT tumour hypoxia (Star et al., 1986, Henderson et al., 1987, Chen et

116 al., 2003). PDT may also lead to vessel constriction via inhibition of the production or release of
117 nitric oxide by the endothelium (Gilissen et al., 1993).

118

119 An important clinical consideration is effective analgesia. In humans PDT produces a
120 sensation of stinging or burning during illumination, especially in sensitive areas such the face, and
121 scalp (Halldin et al., 2011, Chaves et al., 2012). Treatment of large skin areas generally produces
122 more pain than smaller areas (Grapenglesser et al., 2002, Hallidin et al., 2011, Chaves et al., 2012).

123

124 **Photosensitizers for PDT**

125 Photosensitising (PS) agents are natural or synthetic chemicals that transfer light energy to
126 neighbouring molecules, importantly to dissolved oxygen (Allison et al., 2004). Most of the
127 photosensitizers used in cancer therapy are based on a tetrapyrrole structure, similar to that of the
128 protoporphyrin contained in haemoglobin. In clinical practice, a successful PS agent is: nontoxic
129 until light activated, hydrophilic for easy systemic application, activated by a clinically useful light
130 wavelength, and reliably generates a photodynamic reaction (PDR). It also concentrates in tumours,
131 clears normal tissue quickly, and is eliminated from the patient relatively rapidly (Allison and
132 Moghissi 2013a).

133

134 The first-generation photosensitizer, haematoporphyrin derivative (HPD) was a mixture of
135 various monomers, dimers, and polymers of haematoporphyrin (Allison and Moghissi 2013a). The
136 commercially available product, porfimer sodium, marketed under the tradename Photofrin was
137 experimentally used in healthy dogs (Tochner et al., 1991; Panjehpour et al., 1993) and a canine
138 glioma model (Whelan et al., 1993). It was approved for treatment of early stage of human lung
139 cancer in 1998 and for Barrett's esophagus in 2003. The clinical application of Photofrin has been
140 limited by two factors: its absorption peak occurs at 630 nm, too short a wavelength to allow deep

141 penetration of light in tissue. Secondly, Photofrin results in cutaneous photosensitivity lasting up to
142 6 weeks (Zhu and Finlay, 2008).

143

144 These limitations stimulated the development of a second generation of photosensitizers
145 with improved efficiency of ROS generation, more rapid clearance, fewer side effects, and
146 absorption peaks at longer wavelengths (>630 nm red light) where the tissue penetration of light is
147 deeper. One such second-generation photosensitiser is 5-aminolevulinic acid (ALA), a naturally
148 occurring [pro-photosensitiser](#) and precursor for the biosynthesis of heme. For therapeutic purposes,
149 ALA is administered topically (Morton et al., 2008, 2013), orally (Muller and Wilson, 2006), or
150 intralesionally (Hage et al., 2007; Kim et al., 2012) and enters into all cells; although uptake is
151 potentiated by transporters of beta-amino acids and GABA (Rud et al., 2000), highly expressed on
152 some cancer cells and neurons (Zhang et al., 2013). ALA is then metabolised to the red-fluorescent
153 photosensitiser protoporphyrin IX (PpIX, [absorption 635 nm](#)) and finally to non-fluorescent heme
154 (Ajioka et al., 2006, Allison and Moghissi 2013a). This final step relies on ferrochelatase to add
155 Fe^{2+} to PpIX and this rate-limiting enzyme is often deficient in cancer cells (Kemmer et al., 2008).
156 Thus, in the presence of excess ALA, cancer cells that combine high ALA uptake with low PpIX
157 destruction will accumulate PpIX photosensitiser (Collaud et al., 2004). Clinical advantages of
158 ALA treatment include rapid clearance of PpIX from the tissue within 12 hours, resulting in short-
159 lived cutaneous photosensitivity. In human patients ALA has been used for the treatment of T cell
160 lymphoma (Coors et al., 2004), basal cell carcinoma (Kim et al., 2012) squamous cell carcinoma
161 (SCC) and other head and neck cancers (Grant, et al., 1993, Morton et al., 1996). [In veterinary](#)
162 [medicine, ALA has been used to treat SCC in a cow \(Hage et al., 2007\) and in cats \(Bexfield et al.,](#)
163 [2008\), sarcoids in horses \(Gustafson et al., 2004, Golding et al., 2017\) and transitional cell](#)
164 [carcinoma in dogs \(Lucroy et al., 2003a,b\). See Tables 1 and 2.](#)

165

166 The hydrophilic nature of ALA limits its ability to deeply penetrate intact skin and thereby
167 restricts the use of topically applied ALA-PDT to the treatment of superficial diseases, where the
168 tissue structure is disorganised. To overcome this limitation, ALA esters that are less hydrophilic
169 than the parental compound have been developed. The methyl ester of ALA, methyl-
170 aminolevulinate (MAL, Metvix, or Metvixia), was approved by the [US Food and Drug](#)
171 [Administration](#) for PDT treatment of actinic keratosis in 2004 and has shown good results in
172 treatment of [equine sarcoids](#) ([Kemp-Symonds 2012](#), [Golding et al., 2017](#)). Hexaminolevulinate, the
173 n-hexyl ester of ALA, (HAL, Hexvix, Cysview) which is converted to PpIX 50–100 times more
174 efficiently than ALA, was licensed in US in 2010 for the detection of human bladder cancer (Furre
175 et al., 2005). Hexaminolevulinate has also been used intra-operatively in a PDT model in dogs with
176 prostate carcinoma (L'Eplattenier et al., 2008).

177

178 Several other second-generation photosensitisers have been, or are in the process of being
179 developed, each with slightly different origins and characteristics. These include m-
180 tetrahydroxophenyl chlorine ([m-THPC](#), Foscan); 2-(1-hexyloxyethyl)-2-devinyl pyropheophorbide-
181 a (HPPH, Photochlor); palladium bacteriopheophorbide (Padoporfin, [TOOKAD](#)) and its more
182 water-soluble monolysotaurine derivative (Padeliporfin, [TOOKAD-Soluble](#)); motexafin lutetium
183 (Lu-Tex, Lutrin); and Verteporfin (Visudyne). The advantages and indications for these newer
184 agents are summarised in Table 1.

185

186 **Photosensitisers for diagnosis**

187 Photodynamic diagnosis (PDD) uses the fluorescence of photosensitisers to identify tumour
188 tissue in situ. PDD fits within the broader category of Fluorescence Guided Surgery (Allison 2016).
189 The distinction being that, by increasing the illumination intensity or duration, PDD can become
190 PDT. However, whilst the generation of singlet oxygen by photosensitisers is essential for PDT,
191 these same reactive species can damage the photosensitiser and render it non-fluorescent.

ALA has been trialled for PDD in eleven different human tumour types (Nokes, 2013), and is licensed in humans for intraoperative margin assessment in glioma (Hefti et al., 2010, Stummer et al., 2006) and the n-hexyl derivative for bladder cancer (Kausch et al., 2010, Mowatt et al., 2011). Each of the major surgical microscopy and endoscopy manufacturers (Leica, Olympus, Storz, and Zeiss) have specialized imaging equipment for intraoperative PDD for human surgery. Research versions are available for animal models (e.g. Solaris system, Perkin Elmer). However, relatively little work has been done on translating human PDD to veterinary surgery. Veterinary examples include intraoperative cancer imaging and staging in dogs (Knapp et al., 2007, Cabon et al., 2016, Osaki 2016), and image-guided surgery in cats (Wenk et al., 2013). The next generation of agents for photodiagnosis are generally based on near infra-red dyes, which allow deeper views into tissues, sometimes complexed with tumour-targeting peptides or antibodies (Luo et al., 2011, Wenk et al., 2013).

204

205 **Light sources and delivery systems**

The primary requirement when treating lesions with PDT is to ensure that sufficient, homogenous light is delivered to the target tissue. Each PS has an optimal wavelength and intensity (fluence) of light for activation (Sibata et al., 2001). Choice of light source should therefore be based on PS absorption (fluorescence excitation and action spectra), location, size and accessibility of lesions, and tissue characteristics. The clinical efficacy of PDT is dependent on complex dosimetry: total light dose, light exposure time, and light delivery mode (single vs. fractionated or even metronomic). The fluence rate also affects PDT response (Henderson et al., 2006) and as demonstrated in tumour bearing cats by Hahn et al. (1998).

214

The wavelength of light used for PDT is typically in the range between 600–800 nm, the ‘therapeutic window’ (Wilson and Patterson, 1990). In this wavelength range, the energy of each

217 photon is sufficient (1.5 eV) to excite the photosensitizer and yet is low enough to allow the light to
218 penetrate up to 2 cm into the tissue (Zhu and Finlay, 2008).

219

220 The development of light sources and delivery devices with the appropriate dosimetric
221 parameters are key components for the clinical application of PDT. Accurate delivery of the light to
222 the tumour tissue can be accomplished by a variety of light sources and fibre optic delivery devices.
223 Lasers have been one of the main light sources used in PDT. Modern diode lasers are portable and
224 do not require specialized electrical supply or water cooling, providing excellent stability of output
225 power over long periods of time (Mang, 2004). Diode lasers have been approved for use with
226 Photofrin in oesophageal and lung malignancies at 630 nm and at 652 nm for Foscan (Yoon et al.,
227 2013).

228

229 Alternatives to laser technology are non-coherent light sources (Reeds et al., 2004) and light
230 emitting diodes (LEDs), the latter where light is produced by a solid-state process called
231 electroluminescence. LEDs are compact, lightweight and require significantly less energy than
232 lasers. LED systems are capable of output powers up to 150 mW/cm² over a 3 cm x 3 cm area.
233 LEDs have been manufactured with various light output wavelengths, such as 630, 670, and 690
234 nm, which can be used in PDT procedures for flat surface illumination (Mang, 2004 and 2009).
235 Light delivery for treatment of large surface areas, such as treatment of skin diseases, may also be
236 effectively accomplished using broad-spectrum fluorescent lamps (Marcus and McIntyre, 2002).
237 However, LEDs have been shown to be more effective than fluorescent lamps for PDT treatment of
238 squamous cell carcinoma (Novak et al., 2016). One obvious source of light for PDT is the sun, and
239 several recent studies have demonstrated the effectiveness of daylight PDT (reviewed by See et al.,
240 2016). Daylight PDT has obvious potential for veterinary skin cancers, provided the tumour is
241 located where it will be in constant daylight.

242

243 In addition to the light source, delivery devices may be required to provide penetration of
244 light into the target tissue (Star et al., 1992). Fibre-optic devices have been developed for PDT light
245 delivery and dosimetry (Stereborg et al., 2014). The most widely used fibre-optic device in PDT is
246 a cylindrical diffusing fibre tip available in lengths of 1 - 9 cm depending on the specific
247 application. Two light delivery methods have been developed: intraluminal irradiation using light
248 diffusers for the lung and oesophagus, and interstitial illumination methods to deliver adequate light
249 doses to the target tumour volume in head and neck cancers (Yoon et al., , 2013). Fibre optic
250 delivery of PDT has been used in dogs to treat intramedullary bone tumours (Burch et al., 2009).

251

252 **Photodynamic therapy and diagnosis: clinical uses in humans and animals**

253 In contrast, to its increasing use in human medicine, the use of PDT in veterinary medicine
254 has been relatively limited, and although results from small veterinary clinical studies have been
255 published and despite the fact that the dog and cat have been used as a preclinical model in several
256 studies (Lucroy et al., 1999, 2003b, Griffin et al., 2001, Panjehpour et al., 2002, Tanabe et al.,
257 2004), PDT is not well established as a treatment option for tumour bearing animals to date. The
258 main indication currently is in treatment of in situ carcinoma/SCC in cats. Other possible
259 indications are urinary tract neoplasia and glioma in dogs and SCC and sarcoids in horses
260 (Buchholz and Walt, 2013). The following is a comparative review of the clinical experience of
261 application of PDT in human and veterinary medicine to provide a basis for future development and
262 application of the technique in veterinary medicine.

263

264 **Cutaneous tumours**

265 *Carcinoma in situ / Squamous cell carcinoma (SCC)*

266 ALA-PDT is mainly used to treat dermatological cancers in humans and several reviews of
267 current guidelines have been published (Morton et al., 2008, 2013; Wan and Lin, 2014). The results
268 of ALA-PDT in the treatment of human Bowen's disease (squamous cell carcinoma *in situ*) have

269 been promising; randomized, controlled trials comparing ALA-PDT or MAL-PDT to cryotherapy
270 (Morton et al., 1996) or 5-fluorouracil (5-FU) cream (Salim et al., 2003) reveal complete response
271 rates of 82-100% for PDT vs 67-100% for cryotherapy or 79-94% for 5-FU at 12-24 months. The
272 efficacy of topical ALA-PDT in the management of primary cutaneous invasive SCC is variable,
273 with response rates of 54 – 100% reported for superficial lesions and recurrence rates ranging from
274 0 – 69%, but with reduced efficacy in more nodular lesions (Wolf et al., 1993; Morton et al 2002).
275 Current evidence supports the potential of topical ALA-PDT for superficial, micro-invasive SCC
276 but in view of its metastatic potential topical PDT cannot be recommended for invasive SCC
277 (Morton et al., 2008, 2013).

278

279 Cutaneous in situ-carcinoma/SCC in the cat represents the main application for PDT in
280 veterinary medicine to date (Fig. 2). A number of studies have reported response rates from 60 –
281 80+% and disease-free intervals of over 68 weeks, for topical and systemic PDT in cats using a
282 variety of photosensitisers (as detailed in Table 2). As is the case in human patients, the smaller and
283 less invasive tumours respond best to PDT (Magne et al., 1997). PDT has also been used to treat
284 SCC in dogs (McCaw et al., 2000), horses (Giuliano 2008), a cow (Hage et al., 2007), snakes
285 (Roberts WG et al., 1991) and a Great Hornbill (Suedmeyer et al., 2001).

286

287 *Basal cell Carcinoma*

288 PDT has been successfully employed for treatment of basal cell carcinoma (BCC) in human
289 patients as a sole agent or in neoadjuvant setting (Berroeta et al., 2007, Rhodes et al., 2007). A 92%
290 complete response rate was reported with topical ALA-PDT in 330 patients with superficial BCC,
291 but the response rate dropped to 71% in patients with nodular BCC (Zeitouni et al., 2001) , and
292 when topical PDT (with ALA or MAL) is compared to surgery for BCC, PDT consistently shows
293 an increased recurrence rate for both superficial and nodular BCC (Basset-Seguin et al., 2008). This
294 may be due to insufficient penetration of the photosensitizer to deeply located tumour cells when

295 the PS is applied topically. To overcome this problem, the PS may be injected intralesionally.
296 Twenty patients with nodular BCC were treated with ALA in 1% saline solution at estimated dose
297 of 1 mL/cm² injected into the base of tumour. PDT resulted in tumour necrosis, followed by
298 complete re-epithelization after 4-6 weeks with good cosmetic results, no histological evidence of
299 BCC after 3 months and no recurrence during follow-up of 19.5 months (Rodríguez-Prieto et al.,
300 2012).

301

302 Experience of intralesional injection of PS is very limited in animals. One study reported
303 PDT in a cow with ocular SCC using intratumoural injection of ALA. A complete response was
304 observed after 3 months and no relapse 12 months after the treatment (Hage et al., 2007). PDT has
305 also been used for treatment of periocular SCC in horses. A pilot study was conducted using
306 surgical resection plus PDT for periocular SCC in horses by infiltrating wound beds with HPPH
307 prior to illumination. This combination yielded disease-free intervals of 25–68 months. The overall
308 recurrence rate was 22% (2 of 9 horses) and for those horses where local PDT was the first and only
309 treatment modality used, the recurrence rate was 0% (Giuliano et al., 2008).

310

311 *Equine sarcoids*

312 Although of fibroblastic rather than of basal cell origin, equine occult and nodular sarcoids
313 form dermal nodules or plaques and as such bear some physical resemblance to the human nodular
314 BCC. Currently there is no ‘gold standard’ treatment for equine sarcoids, however, PDT has shown
315 promise in the treatment of these common and frustrating lesions. Several small studies have
316 reported encouraging response rates using topical or locally injected ALA or MAL in equine occult
317 and nodular sarcoids. For instance, Gustafson et al., (2004) found a 72% treatment response using
318 ALA-PDT, with recurrence in 39% of lesions after 2 years (n=18). Due to their fibroblastic and
319 bulky nature, cytoreductive surgery may significantly improve response for larger lesions. In one
320 study, CO₂ laser excision with adjunctive MAL-PDT was reported to achieve a 93% one-year

321 disease-free rate (Kemp-Symonds 2012). Most recently, a single application of topical ALA-PDT
322 followed by glycolysis inhibition has been shown to successfully treat equine sarcoids up to 5 mm
323 thick with a 93% response rate ($n=27$) after 1 month, compared with a 14% response rate using
324 ALA-PDT only ($n=7$). Treated sarcoids became scabby with desquamation for 2-4 weeks before
325 healing (Golding et al., 2017) (Fig. 3).

326

327 **Prostate cancer**

328 In humans definitive management of early stage prostate cancer with either surgery or
329 ionizing radiation therapy is associated with significant associated morbidities due to the proximity
330 of normal structures such as nerves, bladder and rectum. By contrast, PDT has the potential to
331 selectively treat the prostate while sparing the surrounding normal tissues because light can be
332 delivered to the entire prostate gland using interstitial cylindrically diffusing optical fibres. Prostate
333 cancer is therefore an attractive target for PDT (Agostinis et al., 2011, Ahmed et al, 2012).

334

335 Vascular-targeted PDT using Padeliporfin mediated PDT and a short drug-to-light interval
336 was shown to carry minimal toxicity in a phase I trial, of prostatic carcinoma patients ($n = 24$) with
337 local failure following radiotherapy (Weersink et al., 2005; Trachtenberg et al., 2007). In a follow-
338 up phase II study, patients were treated with increasing light doses. At 6 months all patients where
339 $>60\%$ of the prostate was determined to be avascular by post-PDT magnetic resonance imaging,
340 had negative biopsies, however, 2 patients (of 28) developed urethrorectal fistulae (Trachtenberg et
341 al., 2008). Following refinement of the technique, a recent phase III randomised controlled study of
342 padeliporfin vascular-targeted PDT (versus active surveillance) has shown this to be a safe and
343 effective treatment for low risk localized prostate cancer (Azzouzi et al., 2017).

344

345 The normal canine prostate has served as a useful preclinical model for evaluating responses
346 to PDT in vivo, since its size and general anatomical structure are similar to those of the human

347 prostate (Waters and Bostwick, 1997). An experimental study was conducted assessing padeliporfin
348 PDT on canine prostate pre-treated with ionizing radiation. All dogs presented normal spontaneous
349 urination upon recovery from the procedure, with no signs of incontinence or significant
350 macroscopic hematuria (Huang et al., 2004). Vascular-targeted photodynamic therapy with WST11
351 (TOOKAD Soluble) has been investigated in a dog model of benign prostatic hyperplasia and was
352 uneventful in all except one dog, which experienced urinary retention. Prostatic urethral width
353 increased as early as 6 weeks after treatment, while prostatic volume decreased, reaching 25% by
354 18 to 26 weeks, this response lasted up to 1 year (Chevalier et al., 2013). Unfortunately canine
355 prostatic carcinoma is not usually detected until symptomatic at which point the disease is in late
356 stage, often with metastatic disease, so it is unlikely that PDT would be beneficial in such patients.

357

358 **Bladder cancer**

359 Photodiagnosis is used in management of human bladder cancers (Mowatt et al, 2011), and
360 bladder cancer is also a potential target for PDT. Human bladder cancers are often superficial and
361 multifocal and can be assessed and debulked endoscopically. Furthermore, the geometry of the
362 bladder allows for homogeneous light delivery via diffusing fibres. In general, early response rates
363 (2 to 3 months) to PDT have been about 50% to 80% of patients with longer-term (1 to 2 years)
364 durable responses in 20% to 60% of patients. It should be noted that many of the patients treated in
365 these studies had recurrent disease that developed after standard therapies such as *Bacillus*
366 *Calmette-Guerin* (BCG) (Agostinis et al., 2011). Treatment of superficial bladder cancer with PDT
367 is generally well tolerated, with dysuria, hematuria, and skin photosensitivity being the most
368 common acute toxicities. Bladder wall fibrosis/diminished bladder capacity can be a problem in
369 some patients (Prout et al., 1987; Uchibayashi et al., 1995). Studies of locally applied (intravesical)
370 ALA demonstrate that comparable complete response rates of 52-60% at 2-3 years can be achieved
371 for patients with treatment refractory bladder carcinoma *in situ* without the prolonged skin
372 photosensitivity experienced using systemic Photofrin (Berger et al., 2003; Waidelich et al., 2003).

373 Despite these promising results, PDT for bladder cancer remains largely investigational with limited
374 use (Agostinis et al., 2011).

375

376 Canine transitional cell carcinoma (TCC) is most commonly located in the trigone region of
377 the bladder precluding complete surgical resection and palliative medical management is often the
378 only treatment available (Fulkerson and Knapp, 2015). PDT could represent a promising option for
379 dogs with TCC. [However, canine TCC is often diagnosed late and is more invasive than human](#)
380 [bladder cancers, making comparisons with human studies difficult \(Fulkerson and Knapp, 2015\).](#) In
381 vitro-studies have shown, that ALA-PDT destroys canine TCC cells (Ridgway and Lucroy, 2003).
382 When studied in vivo, 70% of dogs vomited after oral administration of ALA, but this did not
383 appear to have a negative impact on pharmacokinetics and the active metabolite (PpIX) was shown
384 to accumulate in the bladder mucosa, compared to the muscularis and serosa. Five dogs with TCC
385 of the urinary bladder treated with ALA-PDT and a laser fibre delivery system, showed transient
386 improvement of clinical symptoms with tumour progression free intervals ranging from 4 to 34
387 weeks (Lucroy et al., 2003a,b). The application of PDT for canine TCC clearly warrants further
388 investigation.

389

390 **Brain tumours / glioma**

391 Experimental and clinical studies have demonstrated that PDT can complement current
392 standard therapies (surgical resection, radiation therapy and chemotherapy) in the treatment of brain
393 tumours (Muller and Wilson, 1995, 1996). PDT may be particularly useful as an adjunct to surgery
394 as it can non-invasively target tumour cells infiltrating normal brain. Initial trials provided
395 encouraging results using various formulations of hematoporphyrin derivatives (HPD, Photofrin),
396 ALA as well as mTHPC with light sources including lamps, dye lasers and diode lasers (Agostinis
397 et al., 2011). One of the main indications for ALA in management of glioma is in fluorescence
398 guided surgery (FGS). ALA based FGS has been shown to provide longer survival times than

399 conventional surgery in patients with suspected malignant gliomas ($n=322$), 16.7 versus 11.8
400 months respectively (Stummer et al., 2006).

401

402 In a canine glioma model, dogs were given 0.75 mg/kg Photofrin-II intravenously, followed
403 24 h later by PDT, delivered using a fiberoptic catheter directly to the tumour via a burr hole in the
404 skull (Whelan et al., 1993). This destroyed the tumour without significant brain-stem injury.

405

406 The new classes of PSs, the better understanding of dosimetry and further improvements in
407 technology may significantly change the currently achieved clinical outcome for glioma and other
408 brain tumours both in human and veterinary patients. Pre-clinical data indicating that protracted
409 light delivery may increase the therapeutic index of PDT in the brain combined with newer
410 technologies such as implantable, LED-based light delivery systems could lead to significant
411 improvements in treatment outcomes (Kostron, 2010).

412

413 **Future perspectives**

414 Photodynamic therapy offers great potential due to its selective targeting of tumour cells and
415 minimal normal tissue toxicity. Several innovative strategies have been used to improve PS
416 penetration into tumour cells, including: using an electric current to draw PS deeper into the skin
417 (Lopez et al., 2003), intratumoural PS injection (Hage et al., 2007; Rodríguez-Prieto et al., 2012)
418 and pretreatment with chemical penetration enhancers (Malik et al., 1995; De Rosa et al., 2000;
419 Golding et al., 2017), liposomal formulations and nanoemulsions (Buchholz et al., 2005, 2007).
420 The efficacy of PDT may also be improved by overcoming the antioxidant defences of cancer cells.
421 Antioxidant defences that remove excess ROS are upregulated in many cancers (Tracootham et al.
422 2009), undermining the full potential of PDT. Combination of glycolysis inhibitors with PDT has
423 been shown to deplete cellular antioxidants and significantly improve PDT cytotoxicity against
424 human cancer cells in vitro (Golding et al., 2013) and this combination has proved effective in

425 treatment of equine sarcoids (Golding et al 2017). Other ways in which efficacy of PDT may be
426 improved clinically include: Metronomic PDT (mPDT) to delivery both the drug and light at very
427 low dose rates over an extended period (hours-days) (Lilge et al., 2000), and through use of
428 nanoparticles for PS delivery (Bechet et al., 2008). If the potential for use of PDT in veterinary
429 medicine could be realized this could make a significant contribution to the overall development of
430 the technique.

431

432 **Conclusions**

433 PDT is a safe and effective therapy for many cancers and pre-cancers that can be accessed
434 externally or endoscopically. Small, localised lesions can achieve long-term clearance with
435 negligible scarring or damage to adjacent structures.

436 The science of PDT has seen enormous progress within the past 30 years. For instance: the
437 development of improved photosensitisers, light sources (including endoscopic delivery and
438 daylight PDT), improved understanding of how PDT works, and an expansion of the uses of
439 photosensitisers to allow intraoperative detection of tumour margins. Although PDT has hitherto
440 been used as a monotherapy, the future of the technique undoubtedly lies in combining it with other
441 drugs and approaches as part of a synergistic multimodal treatment.

442 Despite the scientific advances, the clinical practice of PDT is still limited to a small number of
443 individual practitioners or centres of excellence; partly due to a vicious cycle of high photosensitiser
444 costs due to limited demand. With pun intended, veterinary PDT needs to come out of the shadows
445 and into the light. This will only happen if PDT becomes a standard part of the training syllabus and
446 existing PDT practitioners provide internships for the next generation of veterinary surgeons.

447

448 **Conflict of Interest**

449 None of the authors of this paper have a financial or personal relationship with other people or
450 organisations that could inappropriately influence or bias the content of the paper.

451

452 **Acknowledgements**

453 Genilson Fernandes de Queiroz received post-doctoral funding from CAPES (The Coordination of
454 Improvement of Higher Education Personnel – Brazil)

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Table 1.
Summary of characteristics and application of **selected** second generation photosensitizers.

| Agent (synonyms)/ manufacturer | Activation wavelength (nm) | Advantages | Reported tumour applications (human unless stated) | References |
|--|---|---|---|--|
| Foscan (m-tetrahydroxyphenyl chlorine (mTHPC), temoporphin)/ Biolitec Pharma. | 525 - 660 | -Short duration of skin photosensitivity (15 days) -High quantum yield for singlet oxygen -Depth of tumour necrosis (10 mm) | Pleural mesothelioma Head and neck cancers Oesophagus Prostate Pancreas Skin tumours Skin tumours (cats) | Friedberg et al., 2003. Rauschnig et al., 2004; Biel et al., 2006. Lovat et al., 2005; Etienne et al. 2004. Nathan et al., 2002; Moore et al., 2006. Pereira et al., 2007. Triesscheijn et al., 2006. Buchholz et al., 2007. |
| Photoclor (2-(1- hexyloxyethyl)-2-devinyl pyropheophorbide (HPPH))/ AdooQ Bioscience. | 665 - 680 | Extremely hydrophobic, increasing penetration into tissue | Obstructive oesophageal cancer oral squamous cell carcinomas (dogs) facial squamous cell carcinoma (cats) squamous cell carcinoma (horses) | Dougherty et al., 2000. McCaw et al., 2000. Magne et al., 1997. Giuliano et al., 2008. |
| TOOKAD (WST-09, padoporfin, palladium bacteriopheophorbide)/ Steba Biotech. | 760 | New generation photosensitiser with greater stability and short half-life | Prostate (dogs) | Nomura and Mimata, 2012. Huang et al., 2005. |
| Padeliporfin (TOOKAD Soluble, WST-11, palladium bacteriopheophorbide | 760 | Vascular-targeted PDT | Prostate Prostate (dogs) | Azzouzi et al., 2017. Chevalier et al., 2013. |

| | | | | |
|---|---|---|--|---|
| monolysotaurine)/ Steba Biotech. | | | | |
| Lu-TeX (Motexafin lutetium, lutetium texaphyrin/ Pharmacyclics Inc. | 730 | Water soluble. Selectively retained in tumour. Only 24 – 48 h skin photosensitivity | Prostate Rectal (dogs) | Patel et al., 2008. Ross et al., 2006. |
| Talaporphin sodium (aspartyl chlorin, Laserphyrin, Aptocine)/ Meiji Seika Pharma. | 664 - 667 | Retained in tumour for 50 h | Lung. Esophageal. Intranasal (dogs) | Usuda et al., 2007. Yano et al., 2017. Ishigaki et al., 2017. |
| ALA (5-aminolevulinic acid)/ various. Methyl-ALA (MAL, Metvix)/ Galderma. Hexyl-ALA (HAL Hexvix)/ Ipsen. | Pro-drugs. Each metabolized to protoporphyrin IX (414, 635) | Short loading 3 h; short skin photosensitivity 12 h Epithelial penetration improves in sequence ALA>MAL>HAL | ALA: at least 11 different human tumours. Equine sarcoids MAL: basal cell carcinoma. Equine sarcoids. HAL: prostate photodynamic detection. Prostate (dogs) | Nokes et al., 2013. Golding et al., 2017. Morton et al., 2008, 2013. Kemp-Symonds, 2012, Golding et al., 2017. Furre et al., 2005. L'Eplattenier et al., 2008. |
| Verteporfin (Visudyne)/ Novartis | 689 - 693 | Binds low density lipoprotein receptors on abnormal blood vessels and tumours | Wet macular degeneration Esophagus (dogs) Squamous cell carcinoma (horses) | Scott and Goa, 2000. Panjehpour et al., 2002. Giuliano et al., 2014. |

1083 **Table 2.**
 1084 Clinical Reports of photodynamic therapy (PDT) for superficial squamous cell carcinoma (SCC) or
 1085 SCC *in situ* in cats

| Cases / tumour location | PDT agent | PDT method | Response rate / outcome / side effects | Reference |
|--|---|--|--|--------------------------|
| 51 cats Cutaneous SCC facial skin | HPPH-23 Pyropheophorbid- alpha-hexyl-ether | Intravenous administration Argon-pumped dye laser | Overall 61% response rate at 1 year. 100% T1a tumours, 56% T1b and18% T2b. No toxicity, but some morbidity. | Magne et al., 1997 |
| 4 dogs and 4 cats Superficial carcinoma | HPPH | Intravenous administration LED (100 J/cm ² , 33 min) | 8/9 CR >50% PFS > 68 weeks. No cutaneous photosensitivity | Reeds et al., 2004 |
| 13 lesions / cats 10 nasal planum, 2 pinna 1 eyelid | ALA (Cream) | Topical application LED 635 nm 12 J/cm ² | 85% CR rate But with 64% local recurrence, median 21 weeks. Cats attempt to scratch lesion after treatment. Local analgesia required. | Stell et al., 2001 |
| 18 cats with 20 cutaneous SCC | Liposomal formulation of Foscan (m-THPC) | Intravenous administration 625 nm diode laser | 100% CR rate Overall 1 year control rate 75% 20% recurrence, 172 days. Mild erythema/ edema in 15% of cats. | Buchholz et al, 2007 |
| 55 cats Superficial SCC nasal planum | ALA (Cream) | Topical application LED 635 nm 12 J/cm ² | 85% CR rate, 11% PR rate But with 51% recurrence; median 157 days. Transient, mild, local adverse effects. | Bexfield et al., 2008 |
| 12 cats Cutaneous SCC (7 pinna, 2 nasal planum) | Haematoporphyrin derivative (Photogem) | Intravenous administration LEDs (300 J/cm ² 30 min) | No response in invasive tumours or pinna. Small non- infiltrative lesions of nasal planum (n=3) showed CR/PR. One cat developed nasal oedema and died. | Ferreira et al., 2009 |

Abbreviations: LED (light-emitting diode), CR (complete response), PR (partial response), PFS (progression-free survival).

1088 **Figure legends**

1089

1090 **Figure 1.** Fundamentals of photodynamic therapy.

1091 A) Visible and near infra-red light spectrum showing the wavelengths (in nanometres) of maximum
1092 tissue penetration by light (above) and absorbance maxima of selected photosensitisers (below). B-
1093 D) Chemical structures of selected photosensitisers. E) Schematic of photosensitiser mechanism of
1094 action. Photosensitiser (PS) becomes activated (PS*) by light (hν). PS* can undergo two types of
1095 reaction. In Type I reactions, biological material (BM) interacts directly with PS* forming ion
1096 radicals of both species (PS^{•-} and BM^{•+}). BM radical interacts with oxygen and becomes oxidised.
1097 PS radical is either destroyed or reacts with oxygen to regenerate PS and make a superoxide anion
1098 (O₂^{•-}) that can react with BM to oxidise it. In Type II reactions, PS* interacts with oxygen to
1099 regenerate PS and make singlet oxygen (¹O₂), which reacts with BM to oxidise it.

1100

1101 **Figure 2.** Feline nasal squamous cell carcinoma (SCC)

1102 A) An early SCC on the right nasal planum in a Domestic Short-haired cat. B) Application of
1103 photodynamic therapy (PDT) using a high intensity light-emitting diode (LED). C) Complete
1104 resolution of the lesion at 6 weeks, with minimal scar formation.

1105

1106 **Figure 3.** Treatment of equine sarcoids.

1107 A) Painting 5-aminolevulinic acid (ALA) onto sarcoid. B) Application of photodynamic therapy
1108 (PDT). C) Appearance of sarcoid at time of PDT treatment. D) Appearance of sarcoid 1 month after
1109 PDT.